"The Lethal Concentration of Acids and Bases in respect of *Paramœcium aurelia*." By J. O. Wakelin Barratt, M.D., B.Sc., Lond., British Medical Association Research Student. Communicated by Sir Victor Horsley, F.R.S. Received June 15,—Read June 16, 1904.

(From the Physiologisches Institut, Göttingen.)

The present investigation arose out of a research on chemiotaxis, in the course of which it became apparent that a pre-condition of the correct understanding of the nature of chemiotaxis is the quantitative determination of (1) the concentration of the acid and alkaline solutions employed for the study of chemiotactic phenomena, and also of (2) the absolute weight of Paramœcia (or other organisms) added to such solutions. So long as these data are unknown, chemiotaxis can only be investigated qualitatively, and such facts as are ascertainable solely by means of quantitative observations lie beyond the limits of research.

In order that the acid and alkaline liquids employed may be readily comparable one with another, equimolecular solutions are employed in this investigation. The absolute volume of Paramœcia employed in the different experiments was determined by means of the hæmocrit, as in the case of red blood-cells, and from this the weight of Paramœcia was ascertained. In those experiments in which an approximate determination of the weight was sufficient, the Paramœcia were counted, a modification of the method used for the enumeration of red blood-cells being adopted.

In all cases the Paramœcia were obtained in as nearly as possible the same condition. They were removed by centrifugalisation from the liquid in which they had been cultivated, and placed in a large bulk of distilled water for 24 hours before use. At the end of this time they were again concentrated, by centrifugalisation, into a small bulk of fresh distilled water, and were ready for use. In this way contamination of the acid and alkaline solutions employed was avoided, and the modification of chemiotactic reaction brought about by the medium used for cultivation was also, as far as possible, avoided.

The method of investigation adopted for determining the lethal concentration of acids and alkalies consisted in placing Paramœcia in solutions of gradually decreasing molecular concentration, arranged so as to form a geometrical series, each succeeding concentration being half that of the preceding, and noting the time at which death occurred. In all the experiments quoted, in order to make certain that the extremely dilute solutions employed were accurately prepared, their relative conductivity was determined. The latter was measured by the deflection of a sensitive galvanometer, when a fixed potential

difference was established between platinised electrodes immersed in the acid and alkaline solutions employed.

The action of acids and bases upon Paramœcia is shown in Tables I and II. The acids employed are divided into three groups, according to the degree to which they are dissociated; the first consisting of the strong mineral acids, hydrochloric, nitric and sulphuric; the second including the organic acids, formic, lactic, oxalic, tartaric, citric and acetic, together with phosphoric acid; while the third group is made up of extremely weak electrolytes, namely, carbonic, carbolic, hydrocyanic and boric acids. Similarly the bases employed may be arranged in three groups: the first consisting of the strongly dissociated metallic alkalies; the second being represented by the feebly dissociated ammonium hydrate; and the third consisting of the extremely weak electrolyte anilin. In the second column of the tables, the figures in brackets represent the time, in minutes, which elapsed before all the Paramœcia employed were killed. The latter were added in the proportion of about thirty to every 10 c.c. of liquid, and did not appreciably affect the concentration. The temperature of experiment was 16° C. to 18° C.

In 0·0001 N concentration* the strong mineral acids are nearly equally lethal. Some of the weak acids of the second group, in the same concentration, are more lethal than the mineral acids, namely, acetic, lactic and oxalic acids; while others of the same group are less so, namely, phosphoric, citric and acetic. On the other hand the weak electrolytes are lethal in a considerably higher molecular concentration, reaching in the case of hydrocyanic acid 0·3 N.

Since the rate at which chemical change takes place is dependent upon ionic concentration, the dissociation co-efficient calculated from the conductivity (18° C.) or the dissociation constant (25° C.) is given, so far as the available data permit, in the third column of the tables, and the corresponding ionic concentration in the fourth column, the latter being the product of the concentration and dissociation coefficient.

The weak acids are more lethal in less ionic concentration than the strong acids, and the extremely weak electrolytes exhibit the smallest ionic concentration, that of phenol forming the limit of the series. Excluding phenol, however, it is seen that when the acids employed are arranged in the order of their dissociation (Table I), the diminution in ionic concentration proceeds at a much slower rate than the increase of molecular concentration.

The strong alkalies are similarly less toxic than the weak alkali, ammonium hydrate, and the latter again is considerably less so than

* The concentration given in the tables is equivalent, except for carbonic and boric acids, which are regarded as binary compounds, and whose concentration represents gramme-molecules per litre.

Table I.

	Lethal concentration for *Paramœcium aurelia*.	Dissociation coefficient.	Corresponding H^+ ionic concentration.
Hydrochloric acid, HCl	0·0001 N. (50′)	> 0·98	0·000098 N.
Nitric acid, HNO_3	0·0001 (55′)	> 0·99	0·000099
Sulphuric acid, H_2SO_4	0·0001 (40′)	> 0·95	0·000099
Formic acid, HCOOH	0·0001 (11′)	0·75	0·000075
Lactic acid, $CH_3CHOH.COOH$	0·0001 (9′)	0·68	0·000068
Oxalic acid, COOH.COOH	0·0001 (9′)	—	—
Tartaric acid, COOH.CHOH.CHOH.COOH	0·0001 (35′)	—	—
Phosphoric acid, H_3PO_4	0·0001 (85′)	—	—
Citric acid, $\begin{matrix}COOH.CH_2\\ \\ COOH.CH_2\end{matrix}$ COH.COOH	0·0001 (60′)	—	—
Acetic acid, $CH_3.COOH$	0·0001 (85′)	0·30	0·000030
Carbonic acid, H_2CO_3	0·014 (35′)	0·0048	0·000067
Phenol, C_6H_5OH	0·01 (20′)	0·000114	0·00000114
Hydrocyanic acid, HCN	0·3 (15′)	0·000066	0·000020
Boric acid, H_3BO_3	0·225 (60′)	0·000061	0·000014

Table II.

	Lethal concentration for *Paramœcium aurelia.*	Dissociation coefficient.	Corresponding OH^- ionic concentration.
KOH.....	0·002 N. (60′)	0·96	0·00192 N.
NaOH.....	0·002 (40′)	0·98	0·00196
LiOH	0·002 (10′)	—	—
$Ca(OH)_2$.....	0·002 (10′)	0·98	0·00196
$Sr(OH)_2$.....	0·002 (5′)	0·98	0·00196
$Ba(OH)_2$.....	0·002 (5′)	0·998	0·00199
NH_4OH	0·001 (2′)	0·14	0·00014
$C_6H_5NH_2$	0·04 (15′)	0·000108	0·000004

anilin, whose ionic concentration ($4{\cdot}3 \times 10^{-6}$N) is about one five-hundredth of that of lithium hydrate (1960×10^{-6}N) for a nearly equal lethal effect.

The metallic alkalies can be arranged in two periodic groups, the mean lethal concentration of the one (K, Na, Li) being greater than that of the other (Ca, Sr, Ba), when Paramœcia are killed in nearly equal times. Further, the lethal effect runs parallel to the periodic order of these metals, as is exhibited in Table III, in which the atomic

Table III.

Group I.	Death caused by 0·002 N. solution (Table II) in	Group II.	Death caused by 0·002 N. solution (Table II) in
Li, at. wt. 7	10 mins.	Ca, at. wt. 40	10 mins.
diff. 16		diff. 48	
Na, ,, 23	40 ,,	Sr, ,, 88	5 ,,
diff. 16		diff. 49	
K, ,, 39	60 ,,	Ba, ,, 147	5 ,,

weights are given, and the lethal periods repeated from Table II. When the hydrates of calcium, strontium and barium are employed in solutions of weaker concentration, so as to permit of a more accurate determination of the lethal period than is possible when observation extends over so short a period as 5 minutes, it can be shown that strontium hydrate is less toxic than barium hydrate, but the difference between the latter hydrates is much less than that between strontium and calcium hydrates. Similarly the difference in lethal character between sodium and potassium hydrates is much less marked than that between sodium and lithium hydrates. It is not possible, owing to

their insolubility, to employ the remaining members of the above periodic groups in the present investigation.

The considerable difference in ionic concentration both of acids and of bases, for a nearly equal toxic effect, shows that such effect is not hydrolytic in character, for in such a case the concentration of H^+ or OH^- ions would be constant for each series. The relation between periodic grouping and lethal character, exhibited by strong alkalies, supports the view that the latter is dependent upon a chemical reaction not hydrolytic in character.

"Contributions to the Study of the Action of Sea-snake Venoms.—Part I." By Sir Thomas R. Fraser, M.D., F.R.S., Professor of Materia Medica in the University of Edinburgh, and Major R. H. Elliot, I.M.S., on Special Duty for Snake Venom Research, under the orders of the Secretary of State for India. Received May 10,—Read June 9, 1904.

(From the Pharmacology Laboratory of the University of Edinburgh.)

(Abstract.)

The only important contributions to the literature of the subject with which we are acquainted, are those recently made to the Royal Society by Captain Leonard Rogers, I.M.S.

Whilst acknowledging the value of these papers, we desire to state that our work was planned before we saw them, and has in all respects been independent of them.

The venoms used in this research were those of two species of Sea-snakes:—

1. That of *Enhydrina Valakadien* (*a*) expressed in Madras by Dr. Pinto from the venom glands of freshly killed large snakes and sent to us in the dry state, and (*b*) extracted by us from the dried glands of small snakes which had been collected by the same gentleman, and,

2. That of *Enhydris Curtus*, prepared in the same way as the first-mentioned specimen, of which, however, only a small quantity was procured for us by Dr. Pinto.

The Minimum-Lethal Doses of the Various Specimens of Venom.

1. (*a*) Expressed *Enhydrina Valakadien* venom:—

M.L.D. for rats = 0·00009 gramme per kilo. of body weight.
for rabbits = 0·00006 ,, ,, ,, ,,
for cats = 0·0002 ,, ,, ,, ,,